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THE EFFECTS OF NITRIC OXIDE ON MEDIATORS OF CARTILAGE METABOLISM IN HUMAN OA CARTILAGE DETECTED BY ANTIBODY MICROARRAY

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Aim of the Study: In the pathogenesis of osteoarthritis (OA), the course of the destructive process in the cartilage is determined by the balance between anabolic and catabolic mediators, and their regulators. Proinflammatory cytokine interleukin-1 (IL-1) plays a central role in cartilage destruction and the destructive effects of this cytokine are partly mediated by induced nitric oxide (NO) production. In the present study, we investigated secretion of 40 mediators related to cartilage metabolism (e.g. cytokines and destructive enzymes) by 8 OA cartilage samples with an antibody-based microarray. The role of NO on the production of these mediators in OA cartilage was investigated by using an iNOS inhibitor 1400W.
Methods: Cartilage tissue was obtained from the leftover pieces of total knee replacement surgery from patients with OA. Cytokine and NO production was studied in organ culture to allow chondrocytes to live in their natural microenvironment and thus to maintain their characteristic phenotype. Custom Human Cytokine Antibody Array (Ray Biotech, Inc.) was used to measure relative expression levels of 40 cytokines and other inflammatory mediators in the culture medium. Results were considered significant when most of the OA samples showed over 20% change in the relative expression levels when compared to the respective control.
Results: OA cartilage secreted spontaneously 23 out of the 40 measured mediators. Proinflammatory cytokine IL-1β (4 ng/ml) enhanced production of 21 of these inflammatory mediators in OA cartilage along with increased NO production. Inhibition of NO production with a selective iNOS inhibitor 1400W (1 mM) resulted in enhancement of IL-10, IL-17, MMP-13 and TIMP-2 production, while the levels of leukemia inhibitory factor (LIF), MMP-2, MMP-10 and TGFβ 2 were reduced.
Conclusions: OA cartilage produces many of the mediators involved in the pathogenesis of OA. The ability of 1400W to enhance levels of protective IL-10 and TIMP-2 and to reduce levels of destructive LIF, MMP-2 and MMP-10 points to the anti-inflammatory mechanisms that iNOS inhibitors may have in the treatment of OA. However, inhibition of NO production enhanced also the levels of catabolic IL-17 and MMP-13 and reduced the levels of anabolic TGFβ 2.

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INFLUENCE OF COLLAGEN HYDROLYSATE ON THE EXTRACELLULAR MATRIX METABOLISM OF HUMAN CHONDROCYTES

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Aim of the study: Clinical studies have demonstrated the positive effect of orally administered collagen hydrolysate in the treatment of osteoarthritis. The objective of this study was to investigate the effect of a specific collagen hydrolysate (CH) on the biosynthesis of human chondrocytes in a cell culture model.
Methods: The experiments were performed using monolayer cultures and 3D alginate cultures of human femoral head chondrocytes. Human articular cartilage was collected from patients that had undergone primary hip replacement after femoral neck fractures. Presence of osteoarthritis in the operated joint was excluded on the basis of x-ray examinations and patient interviews. Chondrocytes were isolated from the cartilage within 3 hours after surgery and cultured under reduced oxygen conditions.
The CH used in the investigation had a mean MW of 3.3 kDa, with fragments predominantly representing degraded type I collagen.
To start the experiment, culture medium of the isolated chondrocytes was supplemented with CH and at the end of the observation period type II collagen biosynthesis was quantified by means of ELISA technique. The results were confirmed by immunocytochemical detection of type II collagen and by analyzing the incorporation of ^{14}C-proline into matrix proteins. Moreover, the amount of pericellular proteoglycans was determined by a colorimetric assay and protease activity in the culture media was assayed by means of gelatine-substrate zymography.
Results: Supplementation of the culture medium with CH led to a dose-dependent, statistically significant ($p<0.05$) increase of type II collagen biosynthesis in human chondrocytes compared to the control cells cultured in basal culture medium. At a concentration of 0.5 mg CH/ml a maximum stimulation of type II collagen synthesis of more than 25% was observed. Moreover, the amount of pericellular proteoglycans was also significantly ($p<0.05$) increased after administration of CH, whereas the presence of extracellular CH had no significant effect on the protease activity of human chondrocytes compared to the untreated controls.
Conclusion: These results indicate a stimulatory effect of CH on the synthesis of extracellular matrix macromolecules by human chondrocytes, whereas protease activity of the cells is not affected by CH treatment. Based on these this data, we have provided evidence that CH might contribute to reduce degenerative alterations of the extracellular matrix and thus might be of therapeutic relevance in the treatment and prevention of osteoarthritis.

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THE TYPE I NITRIC OXIDE SYNTHASE IS EXPRESSED IN NORMAL BUT NOT IN OSTEOARTHRITIC HUMAN CHONDROCYTES

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Aims: To compare the expression of the type I nitric oxide synthase (NOS I) in normal and osteoarthritic human chondrocytes and to study its regulation by anabolic growth factors and pro-inflammatory cytokines.
Methods: Chondrocytes were isolated from the cartilage of the femoral condyles obtained from 8 osteoarthritis (OA) patients undergoing total knee replacement surgery and from 2 cadaveric donors without macroscopic signs of arthritis. After isolation, chondrocytes were plated in 6-well plates at high density to prevent dedifferentiation. NOS I protein levels were evaluated in the cytoplasmic extracts by Western blot with a rabbit anti-human NOS I antibody (Abcam Ltd., Cambridge, UK), followed by a goat anti-rabbit IgG-alkaline phosphatase-conjugated antibody. Immunoreactive complexes were detected by chemifluorescence using the ECF reagent (Amersham Biosciences, Buckinghamshire, UK). Fluorescence was detected with a Storm 840 fluorescence scanner (Amersham Biosciences).
Results: NOS I protein was detected in the cytoplasmic extracts from the normal donors and from one OA sample. The other 7 OA samples had no detectable NOS I protein. Treatment of the